A polymerase chain reaction assay of cerebrospinal fluid in patients with suspected herpes simplex encephalitis

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Abstract

A polymerase chain reaction (PCR) was used to detect herpes simplex virus (HSV) deoxyribonucleic acid (DNA) in CSF of 109 patients with possible herpes simplex encephalitis. HSV DNA was found in 20/109 patients. In 14 of these patients the diagnosis was confirmed by a rise in CSF antibodies, isolation of HSV from the brain, or both. In 3 patients CSF antibodies did not rise and 3 patients did not have a follow up lumbar puncture or a brain biopsy. In 19/20 patients HSV DNA was present in the first CSF specimen. The virus was identified as HSV I in 15 patients and HSV II in 4; the virus was not typed in the other patient. A possible diagnosis of herpes simplex encephalitis was not confirmed in the 89 PCR-negative patients. HSV DNA was present in CSF of 3 patients who had meningitis with herpetic genital infections but it was not found in 24 patients with other neurological diseases. The results suggest that the detection of HSV DNA in CSF using a PCR assay will be an accurate method of early diagnosis of herpes simplex encephalitis.

(*J Neurol Neurosurg Psychiatry* 1993;56:520-525)

A safe and accurate method of early diagnosis of herpes simplex encephalitis has been an elusive goal. Herpes simplex encephalitis characteristically causes a severe encephalopathy with focal neurological symptoms and signs, but the clinical manifestations can be mimicked by other diseases.¹ Abnormalities on EEG,² CT³ and MRI⁴ are common but they are not specific for herpes simplex encephalitis. A rise in the titre of antibodies to herpes simplex virus (HSV) in the CSF may confirm the diagnosis of herpes simplex encephalitis but HSV antigens and antibodies are rarely detectable in the early stages.⁵⁻⁷

The most accurate method of diagnosing herpes simplex encephalitis has been the isolation of HSV or viral antigens from brain tissue,⁸ but a brain biopsy has a risk of complications,⁹ it may miss a diagnosis of herpes simplex encephalitis¹⁰ and it can provide misleading information.¹¹ Furthermore, transportation of a seriously ill patient with encephalitis to a hospital that has a neurosurgical unit is risky. Many clinicians prefer to treat patients with acyclovir without confirming the diagnosis of herpes simplex encephali-

tis but this approach runs the risk of overlooking other diseases.¹

HSV can be isolated from CSF cultures in only 4% of patients with herpes simplex encephalitis.⁵ The low rate of detection of HSV may be a result of the small quantity of virus in the CSF or neutralisation of infectious virus by antibody. Virus particles that are complexed with antibody retain their nucleic acid component. The polymerase chain reaction (PCR) can amplify small quantities of selected deoxyribonucleic acid (DNA) sequences until they are detectable by conventional DNA hybridisation.¹² Evidence from small groups of patients and studies using stored CSF samples from patients with proven herpes simplex encephalitis suggests that identification of HSV DNA in CSF using the PCR may be a useful method of diagnosing herpes simplex encephalitis.¹³⁻²⁰ However, the place of the PCR assay for HSV DNA in CSF in ordinary clinical practice is uncertain.²¹

Patients and methods

From 1989 CSF has been obtained from 109 patients with a clinical diagnosis of possible herpes simplex encephalitis. A total of 38% of the patients were in Auckland Hospital and 62% were in other hospitals in Auckland, other parts of New Zealand and the Pacific Islands. CSF specimens were also obtained from 3 patients with acute meningitis and herpetic genital infection, and 24 patients with other neurological diseases: rubella encephalitis 6, disseminated varicella-zoster infections 7, benign intracranial hypertension 3, cerebrovascular disease 2, collagen vascular disease 1, Guillain-Barré syndrome 1, chronic steroid-responsive meningitis 1, syringomyelia 1, multiple sclerosis 1, positive serum syphilis serology 1. Whenever possible, simultaneous blood samples were obtained. Specimens were stored at 4°C until used. Each CSF and blood specimen was tested for HSV DNA using PCR (see below) and HSV antibody by complement fixation assay. When there was enough CSF the integrity of the blood-brain barrier was assessed. Aliquots of CSF and serum (minimum volume 250 µl) were analysed in a Behring Nephelometer (Behringwerk AG Diagnostica D3550 Maburg, Germany) for both albumin and total IgG content and the CSF/serum albumin index was calculated.²² Intrathecal antibody synthesis in CSF samples containing

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Received
28 September 1992
and in revised form
23 November 1992.
Accepted
27 November 1992

HSV antibody was confirmed by calculating the CSF IgG index.²²

Polymerase chain reaction

An aliquot of CSF was added to an equal volume of 2x lysis buffer, up to a maximum volume of 500 µl. Final concentration of the digestion solution was 10mM Tris-hydrochloride (Tris HCl) (pH 7.6), 10mM ethylene diamine tetraacetic acid (EDTA), 10mM sodium chloride, 0.5% sodium dodecyl sulphate and 100mg/ml Proteinase K (Boehringer Mannheim).

Blood leucocytes were prepared by separating 5 ml of EDTA blood on a Ficoll gradient consisting of 2 ml of Ficoll-Hypaque (density 1.12) overlaid with 3 ml of Ficoll-Paque (density 1.077) (Flow Laboratories). Both lymphocytes and polymorphonuclear cells were harvested, washed twice in phosphate-buffered saline (pH 7.4), suspended in 1ml phosphate buffered saline and aliquoted into lysis buffer as above.

Samples were incubated at 55°C for 16 hours. DNA was recovered from the CSF or blood leucocytes by phenol/chloroform extraction followed by ethanol precipitation. The concentration of DNA in extracted blood leucocytes was determined by measurement of optical density at 260nm. Approximately 1 µg of DNA was added to each PCR reaction. The amount of DNA in CSF was not quantifiable and an extracted

sample equivalent to 50 µl CSF was added to a reaction mixture containing 16.6mM ammonium sulphate, 67mM Tris HCl (pH 8.8), 6.7mM magnesium chloride, 10mM β-mercaptoethanol, 6.7mM EDTA, 1.5mM each of dATP, dCTP, dGTP, dTTP and 170 µg/ml of bovine serum albumin (Sigma). Two units of Taq polymerase (Cetus Corporation) were added to each 50 µl reaction mixture. The primer sequences were chosen to flank a conserved region in the HSV I and II glycoprotein D gene.¹³ PCR reactions were sequentially subjected to denaturation for 3 minutes, annealing at 60°C for 30 seconds, chain elongation at 65°C for 2 minutes and denaturation at 95°C for 1 minute. Forty cycles of amplification were performed. Positive control DNA was prepared from HSV-infected human embryo fibroblasts. To monitor for potential contamination, aliquots of lysis buffer with no added DNA were processed and amplified in parallel with each batch of patient samples.

Serial dilutions of purified genomic DNA showed this assay was able to detect 10² viral genome equivalents. HSV I and II amplified with equal efficiency.¹³ The identity of the 142 base-pair HSV I and II specific PCR product was confirmed by Southern blotting and hybridisation with a ³²P-labelled oligonucleotide probe derived from an internal region of the amplicon with complete sequence homology between the two HSV types: AGC-GCC-GTC-AGC-GAG-GAT-AAC-CTG-GG. Individual virus strains were typed by restriction endonuclease digestion of the amplified product. Each of the 20 base-pair primers targeted a region of virus genome that was completely conserved between HSV types I and II. Virus genome encompassed by the two primers also was highly conserved with less than 6 base-pair variation between types I and II. Despite this close homology unique restriction sites were identified so that amplified product could be typed by digestion with *HpaII* and *HinfI* (fig 1).

Separate facilities and laboratory equipment were used for PCR reagent handling, DNA extraction and gel electrophoresis. Laboratory personnel with active herpes labialis were not involved in sample processing or the PCR.

Results

HSV DNA was detected in the CSF from 20 of the 109 patients with a clinical diagnosis of possible herpes simplex encephalitis (table 1). There was other laboratory evidence of herpes simplex encephalitis in 14 of these patients. In 4 patients HSV was isolated from brain tissue and there was an increase in HSV antibody titres in the CSF in the other 10. These 14 patients had an acute encephalopathy and a CSF pleocytosis. Focal neurological symptoms and signs were present in 12/14 patients and seizures occurred in 10. The 2 neonates were afebrile but fever was present in the other 12 patients. A CT scan was obtained in 13 of these patients and in 8 it

Figure 1 Restriction digestion of the HSV-specific amplification products. Pedigree HSV-I and HSV-II strains were amplified to demonstrate the strain-specific restriction pattern of the amplified product. 5 µl aliquots of each completed PCR reaction were subjected to restriction enzyme digestion with *HinfI* and *HpaII*. A specific recognition sequence for *HpaII* restriction endonuclease is only present in the amplification product derived from HSV-I DNA. The recognition sequence for *HinfI* is present only in the amplification product derived from HSV-II DNA. Products were electrophoresed on 2% agarose gel. Arrow marks position of undigested amplification product. HSV-I derived amplification product: (a) not digested; (b) digested with *HinfI*; (c) digested with *HpaII*. HSV-II derived amplification product: (d) not digested; (e) digested with *HinfI*; (f) digested with *HpaII*. (M): Molecular weight markers (*HpaII* digest of pBR322).

Table 1 Patients with HSV DNA in CSF

Patient	Age	Sex	Focal signs	CSF WBC (10 ⁶ /l)	CT ¹	EEG	Diagnostic confirmation (Ab=Ab in CSF)	HSV type	Positive CSF viral culture	Follow up PCR ²	Number of days treated	Residual neurological deficit ³
1 ⁴	18	F	+	196	+	Generalised abnormality, focal accentuation	Ab<1:4->1:32	II	0	4+, 11+, 18-	20	Mild
2	16	M	+	194	N/D	N/D	Ab 1:4->1:32	I	0	37-	14	Mild
3	46	F	+	512	+	N/D	Brain biopsy Ab<1:4->1:16	I	N/D	7-	14	Mild
4	62	M	+	208	+	Generalised abnormality, focal epileptiform	Autopsy Ab 1:64	I	0	N/D	3	Dead
5	70	F	+	215	0	N/D	Ab<1:4->1:32	I	N/D	35-	8	Severe
6	53	F	+	30	0	PLEDS	Brain biopsy	I	0	12-	12	Mild
7	69	M	+	134	+	Focal epileptiform		I	0	N/D	3	Dead
8	32	F	0	420	0	Generalised abnormality, focal epileptiform	Ab<1:4->1:64	I	0	8-	16	Complete recovery
9	3 weeks	F	0	31	0	N/D	Ab<1:4->1:32	II	0	14+, 20+, 27-, 34-	28	Dead
10	6	F	+	18	+	Generalised abnormality, epileptiform	Ab 1:32	I	N/D	9+, 16-	21	Mild
11	51	M	+	380	+	Generalised abnormality, focal epileptiform	Brain biopsy Ab 1:8	I	N/D	N/D	19	Dead
12	67	M	+	88	+	N/D		I	N/D	35+ (weak)	21	Mild
13	2 weeks	M	+	163	0	Generalised abnormality, multifocal epileptiform	Ab<1:4->1:4	II	ND	11+, 22-	21	Mild
14	14	F	+	50	+	N/D	Ab 1:8	I	N/D	8+, 21-, 54-	21	Mild
15	1 week	M	0	2	N/D	N/D	0 ⁵	II	0	N/D	0	Dead
16	69	F	+	5	+	PLEDS	0 ⁵	I	N/D	N/D	10	Mild
17	65	F	+	14	0	Generalised abnormality, PLEDs	0 ⁵	I	N/D	N/D	2	Dead
18	43	F	0	66	+	Generalised abnormality (mild)	0	I	0	14+ (weak)	18	Severe
19	40	F	0	60	0	Generalised abnormality	0	N/D	0	20-	10	Dead
20	59	F	0	98	0	PLEDS	0	I	N/D	4+, 13-	14	Complete recovery

CT¹ :+ = low attenuation lesion in one or both temporal lobes ± contrast enhancement or haemorrhage.

Follow up PCR² :numbers refer to days after first lumbar puncture.

Residual neurological deficit³ :mild = symptoms and signs without impairment of activities of daily living; severe = severe symptoms and signs with loss of independence.

⁴ :previously reported patient¹³.

⁵ :follow up lumbar puncture not done.

Abbreviations

:M = male.

:F = female.

:N/D = not done.

:PLEDS = Periodic lateralised epileptiform discharges.

:CSF WBC = cerebrospinal fluid white blood cells.

:CT = computerised tomography.

showed an area of low attenuation in one or both temporal lobes which in 3 cases, was accompanied by patchy contrast enhancement. In one of the neonates (Patient 13) there was low attenuation throughout the cerebral cortex and meningeal enhancement. The CT scan was normal in 4 patients.

Confirmation of the diagnosis was not possible in the other 6 patients who had HSV DNA in the CSF. Three of these patients (patients 15-17) did not have a follow up lumbar puncture or a brain biopsy. Patients 16 and 17 presented with an acute encephalopathy, fever and focal neurological signs and the EEG showed periodic lateralised epileptiform discharges. The CT scan in patient 16 was typical of herpes simplex encephalitis. Patient 15 had meconium aspiration and seizures at birth. After a brief improvement abnormal movements appeared and prolonged seizures recurred. The patient died at 16 days. The CSF cell count was normal and bacterial and viral cultures were negative. There was insufficient CSF to measure HSV antibodies and a second CSF sample was not obtained. A necropsy was not carried out.

In three patients with HSV DNA in the CSF (patients 18-20) the antibody titre in the CSF did not rise. Patient 18 had been receiving chemotherapy for disseminated malignant melanoma when she presented with a 12 hour history of headache, fever, confusion and drowsiness. The CSF leucocyte count was 66x10⁶/l (100% lymphocytes)

and CT showed low attenuation in both temporal lobes. HSV DNA was present in the first CSF specimen and was still detectable 14 days later. A brain biopsy was not obtained.

Patient 19 presented with headache, fever, drowsiness and confusion over 2 weeks. There were no focal neurological signs. The CT was normal but the CSF leucocyte count was 60x10⁶/l (71% lymphocytes). A weak HSV DNA signal was found only in the second of 3 CSF specimens but HSV antibodies were not detected in the CSF. A frontal lobe biopsy was obtained 10 days after acyclovir had been started. There were scattered areas of lymphocytic infiltration, but HSV was not isolated. The patient died 4 weeks after admission.

Patient 20 developed fever, headache, complex partial seizures, abnormal behaviour and a lymphocytic CSF pleocytosis. The CT scan was normal but the EEG contained delta and sharp waves in the left frontotemporal area. The patient recovered after treatment with acyclovir.

HSV DNA was found in the first CSF specimen in 19/20 patients. In patient 19, HSV DNA was found only in the second sample. The virus was identified by restriction endonuclease digestion of the amplified product as HSV I in 15 patients and HSV II in 4 (table 1). In one patient the virus was not typed. Further CSF samples were obtained in 14/20 patients (table 1). Three patients had another lumbar puncture within a week of

Figure 2 Persistence of signal in the CSF in Patient 9. Equal volumes of extracted material (equivalent to 50µl of CSF) were amplified from sequential CSF samples from a neonate with disseminated HSV infection. The HSV-specific amplification product was present in the CSF on day 1 (a), day 14 (b) and day 20 (c) and absent on days 27 (d) and 34 (e). No DNA control (f). Positive control (g). Arrow shows 142 base-pair specific amplification product.

starting treatment and in one of these cases HSV DNA had disappeared after 7 days. In 7 patients HSV DNA was still present in the CSF 8 to 35 days after the first lumbar puncture (fig 2) and in 5 patients it persisted beyond 10 days. HSV DNA was detected in blood leucocytes in one of the 5 patients tested (patient 18). In 9 patients HSV was not isolated from CSF viral cultures. The CSF was not used for viral cultures in the other 11 patients.

HSV DNA was not found in the CSF of

Table 2 Patients with initial clinical diagnosis of possible herpes simplex encephalitis but negative HSV PCR: Final Diagnoses

Diagnosis	Number
Other CNS infections	13
Parainfectious encephalomyelitis	5
Viral meningitis	4
Epilepsy	4
Idiopathic infantile apnoea	3
Cerebrovascular disease	3
Other diagnoses	16
Encephalopathy of unknown aetiology (see table 3)	41

the other 89 patients with possible herpes simplex encephalitis. A final diagnosis was reached in 48 of these patients (table 2). The cause of the acute encephalopathy was not found in the other 41 patients (table 3). None of the patients who had an acute encephalopathy of unknown cause had a brain biopsy and HSV antibodies did not appear in the CSF in the 6 patients who had another lumbar puncture after 10 days or more. Ten of 41 patients without a final aetiological diagnosis had focal symptoms and signs with a CSF pleocytosis. The CT was typical of herpes simplex encephalitis in only one of these patients. Thirteen patients had a non-focal encephalopathy with a CSF pleocytosis. Only 2 of these patients presented with stupor or coma and CT scans were either normal or showed a non-specific abnormality. There were focal symptoms and signs and a normal CSF in 5 patients, all of whom had an acute onset of repeated epileptic seizures and post-ictal stupor or coma. The CT findings were not suggestive of herpes simplex encephalitis. Two of these patients had a poor recovery: one was admitted to a hospital outside New Zealand and did not have a CT scan, and the other did not have HSV antibodies in the CSF. Thirteen patients had a non-focal encephalopathy with a normal CSF.

Table 3 Clinical features, investigations and outcome in patients with encephalopathy of unknown cause

	Encephalopathy with CSF pleocytosis		Encephalopathy without CSF pleocytosis	
	Focal (%)	Non-focal (%)	Focal (%)	Non-focal (%)
Number	10	13	5	13
Fever	9 (90)	10 (77)	5 (100)	6 (46)
Focal symptoms/signs	10 (100)	0	5 (100)	0
Seizures	6 (60)	1 (8)	5 (100)	2 (15)
Mental State				
Drowsy/confused	6 (60)	11 (85)	0	12 (92)
Stupor/coma	4 (40)	2 (15)	5 (100)	1 (8)
CT Scan				
Normal	5/9	10/11	3/4	7/9
Typical HSE	1/9	0/11	0/4	0/9
Other abnormality	3/9	1/11	1/4	2/9
Electroencephalogram				
Normal	1/7	1/8	0/4	1/6
Mild/mod abnormality	0/7	4/8	2/4	2/6
Severe generalised	2/7	2/8	1/4	3/6
Focal abnormality	4/7	1/8	1/4	0/6
Rise CSF HSV Ab ¹	0/3	0/2	0/1	0/0
Treatment acyclovir	7 (70)	7 (54)	3 (60)	6 (46)
Outcome				
Complete recovery	7 (70)	11 (85)	2 (40)	11 (85)
Mild deficit ²	3 ⁴ (30)	2 (15)	0	1 ⁴ (8)
Severe deficit ³	0	0	2 (40)	0
Died	0	0	0	0
Not known	0	0	1 (20)	1 (8)

CSF HSV Ab¹ : cerebrospinal fluid herpes simplex virus antibody.
Mild deficit² : symptoms, signs but no impairment of activities of daily living.
Severe deficit³ : severe symptoms, signs with loss of independence.
⁴ : incomplete follow up.

HSV DNA was detected in the CSF of 3 patients who had an acute meningitis and herpetic genital infection, although the virus was not isolated from CSF cultures. CSF specimens from the control patients with other neurological diseases did not contain HSV DNA.

Discussion

The PCR is a highly specific and sensitive method of detecting DNA.¹² The DNA primers used in our assay are specific to HSV and will not detect DNA from other herpes viruses.¹³ Specific DNA sequences can be amplified by a factor of 10^6 within a few hours, but the exquisite sensitivity of the PCR also is its main potential drawback.²³ Contamination with amplified DNA product in the laboratory is a well recognised problem and standard procedures now include segregation of sample preparation and amplification, use of positive displacement pipettes and inclusion of multiple assay blanks with each run.

Many individuals harbour HSV and intermittently excrete the virus.²⁴ Samples of CSF may be contaminated by medical, nursing or laboratory staff. The risk of external contamination is increased when specimens are obtained at another hospital, because they will be handled by two sets of laboratory staff. A total of 62% of our samples were sent from other hospitals and we expected that if external contamination was a problem, it would show up in this study. In the presence of a viraemia, CSF may also be contaminated by blood leucocytes during a traumatic lumbar puncture. Latent HSV infection of the brain in individuals who do not have herpes simplex encephalitis is another potential source of false-positive results.¹⁶

Other laboratory tests supported the diagnosis of herpes simplex encephalitis in 14/20 patients with HSV DNA in the CSF. A diagnosis of herpes simplex encephalitis was not confirmed in the other 6 patients, although the clinical picture, CSF and CT suggested this was the correct diagnosis. In 3 of these patients a second CSF specimen was not examined and a brain biopsy was not obtained. In the other 3 patients, antibodies to HSV did not appear in the CSF. CSF antibodies to HSV may not appear in biopsy-proven herpes simplex encephalitis⁸ and in patient 18 antibody production may have been blunted by chemotherapy. The diagnosis of herpes simplex encephalitis was most doubtful in patient 19, since HSV DNA was detected only in the second of 3 CSF specimens and HSV was not isolated from the brain biopsy. Nevertheless, a brain biopsy misses the diagnosis in 4% of patients with untreated herpes simplex encephalitis¹⁰ and a false-negative biopsy may be more common if acyclovir has been started.

As far as we know there were no false-negative PCR assays in the patients with a clinical diagnosis of possible herpes simplex encephalitis, but the sensitivity of the assay

cannot be assessed accurately in our patients. None of the PCR-negative patients had a brain biopsy, which is the "gold standard" for the diagnosis of herpes simplex encephalitis. Since the introduction of acyclovir, many clinicians prefer to treat suspected herpes simplex encephalitis without confirming the diagnosis with a brain biopsy. Retrospective confirmation of the diagnosis by measurement of CSF antibodies is often not sought if the patient is recovering. CSF antibodies have been used as the "gold standard" for the diagnosis of herpes simplex encephalitis in most of the patients in other studies using the PCR assay for the detection of HSV DNA in CSF.¹⁵⁻²⁰ The sensitivity of the PCR assay compares favourably with CSF antibody measurement. Until the PCR assay is compared with brain biopsy in a large number of unselected patients with suspected herpes simplex encephalitis, its true sensitivity will be uncertain.

HSV DNA occasionally has not been found in the CSF after early treatment with acyclovir.¹⁷ We found that HSV DNA usually was present in the CSF for one to two weeks after treatment was started, and it sometimes persisted for longer. In one patient a weak signal was still present after 35 days. Other investigators found that HSV DNA persisted in the CSF for up to 27 days,^{16 17 20} but it usually disappeared within 14 days.^{17 18}

Another possible cause of a false-negative result is failure of the primers to recognise both HSV I and II. Neonatal herpes simplex encephalitis can be caused by HSV I or II.²⁵ HSV I is the cause in the overwhelming majority of children and adults with herpes simplex encephalitis²⁵ but, as in patient 1, HSV II occasionally is the causative agent in adults. If the primers used in the PCR assay recognise only HSV I DNA, HSV II will not be detected. This potential source of false-negative results was not a problem in our patients because the primers identified both types of HSV.

A third potential reason for a false-negative assay is "genomic drift" of the virus. Although HSV is genetically relatively stable, base substitution does occur.²⁶ Some strains of HSV may be sufficiently diverse to escape primer detection.

The detection of HSV DNA in CSF by the PCR can be used as a guide to early treatment. In 19/20 patients HSV DNA was found in the first CSF specimen. Others found that HSV DNA occasionally was not present in the first CSF sample but a second specimen collected 24 hours later was positive.^{17 18} The PCR assay itself is rapid and can provide a result within 24 to 48 hours.

Most clinicians continued acyclovir until HSV DNA had disappeared but it is not yet known if persistence of HSV DNA in the CSF is a useful guide to the duration of treatment. In 5 patients HSV DNA persisted beyond 10 days. All of these patients received a prolonged course of treatment and 3 had a satisfactory recovery. Other investigators have found that a relapse of herpes simplex

encephalitis sometimes was heralded by the reappearance of HSV DNA in the CSF.^{18 20}

The cost of materials for a single PCR reaction is approximately \$NZ15 (£5). A high level of technical skill is required for reliable performance of the assay and supervision by an experienced molecular biologist is recommended. The test may therefore be available only in large hospitals. If the PCR assay for HSV DNA is not available locally, CSF can be sent to a laboratory that performs the test. Our experience suggests that contamination is not a significant problem when CSF is transported from other hospitals.

Our results indicate that the detection of HSV DNA in the CSF with a PCR assay is a highly specific test for herpes simplex encephalitis. The assay should detect small quantities of HSV DNA but a precise estimate of its sensitivity cannot be provided, because most patients in this and other studies did not have a brain biopsy. We believe that the PCR assay has a useful role in the early diagnosis and treatment of herpes simplex encephalitis, while a brain biopsy usually can be avoided. Acyclovir should be started immediately if the history, examination, CSF, EEG, CT scan or MRI scan suggest that herpes simplex encephalitis is a possible diagnosis. If HSV DNA is present in the CSF, a full course of acyclovir is required. A negative result should be interpreted with caution but it suggests that herpes simplex encephalitis is not the correct diagnosis and an alternative diagnosis should be sought. Unless the clinical, EEG and neuroimaging findings strongly support herpes simplex encephalitis, it is reasonable to stop acyclovir if the PCR assay is negative.

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